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STORAGE

THE GENUS *RHIZOCTONIA* IN INDIA

BY

F. J. F. SHAW, B.Sc. (Lond.)

AND

S. L. AJREKAR, B.A., Dip. Agr. (Cantab)

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AGRICULTURAL RESEARCH INSTITUTE, PUSA

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F. J. F. SHAW, B.Sc. (Lond.),

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THE genus *Rhizoctonia* has attracted considerable attention in recent years in India, Europe and, particularly, in America. The previous work[1] in this country was concerned chiefly with the form *R. Solani* Kühn, which was shown to be the cause of extensive damage to jute, cotton, and other crops. Moreover cross inoculations between different hosts revealed the presence of a specialization in the parasitism of this fungus analogous to that so well known in *Puccinia* and *Erysiphe*. The present paper describes two species of *Rhizoctonia* of some economic importance in India and presenting a few points of general scientific interest. It is unnecessary to recapitulate here the historical information relating to this genus which has been summarized in a previous communication.[1]

Rhizoctonia Napi West.

In January 1914, the state of the mustard crop in one field of the Pusa Farm indicated the presence of a virulent disease. The crop was about three feet high and was well developed, but the stems, pods, leaves, in fact all parts of the plant, were covered with a thick white growth of mycelium. This produced a dry rot at the nfected area and as a result a large number of plants were lying prone on the ground with bent and broken stems. In the majority of cases the patches of mycelium contained sclerotia. These were large white aggregations of hyphæ, which ultimately turned black on the outside; they were common in the cavity of the pith and inside the pods. The striking point about this parasite was its omnivorous nature; in fact

1 Shaw, F. J. F. "Morphology and Parasitism of Rhizoctonia," *Mem. Dept. Agri. India, Botanical series*, Vol. IV, No. 6, 1912.

the fungus appeared to spread upon all living plants in the vicinity of the mustard as the following list of hosts will show :—

Amaranthus tristis
Argemone mexicana
Avena sativa
Beta bengalensis
Brassica campestris var. *glauca*
Calamintha
Cannabis sativa
Chenopodium album
Cicer arietinum
Cincus arvensis
Fumaria parviflora
Hordeum vulgare
Lathyrus sativus
Lens esculenta
Leucas
Linum usitatissimum
Medicago lupulina
Pisum sativum
Scoparia dulcis
Triticum vulgare
Vicia hirsuta

A second outbreak of this disease took place in February 1915, when a small field of gram was attacked. In this case the fungus occurred chiefly on the pods (Pl. I, fig. 2.), which turned white and became filled with the fungus. The same parasite has also been found attacking cruciferous plants (e.g., *Erysimum*) in gardens in which the soil had been heavily manured with cattle manure, and the fact that the field of mustard on which it first appeared in Pusa had also received heavy applications of organic manures suggests that the outbreak of the disease is favoured by this treatment. The infection might pass into the soil with the manure or the latter may merely create the conditions which are necessary for the rapid development of a fungus hitherto dormant in the soil ; the lowering of the power of disease resistance in a crop which results from heavy nitrogenous manuring is well known.

Inoculations upon glucose agar medium[1] resulted in a rapid growth of the fungus and the production of numerous sclerotia (Pl. VI, fig. 3). The hyphæ were of the typical *Rhizoctonia* form, the branches of the mycelium being characterized by the basal constriction and transverse wall (Pl. V, fig. 1) so well known in other species ; the cells were about 160μ long and 16μ broad but showed great variation in size. The sclerotia were irregular round bodies black on the outside and white in the interior (Pl. II, figs. 2, 3); they were about

[1] Extract of Lemco .. .. 4 grms.
Sodium chloride .. .. 5 „
Peptone .. .. 10 „
Glucose .. .. 20 „
Agar .. .. 15 „
Water .. .. 1000 c.c.

2 to 5 mm. in diameter, but by several sclerotia adhering together composite bodies up to 1 or 2 cm. may be formed. The sclerotia consist of hyphæ tightly woven together, of which the outer two or three layers have divided up into small thick-walled black rectangular cells forming a protective covering to the softer tissues of the interior (Pl. II, fig. 3). A comparison of the sclerotia and hyphæ with the known species of *Rhizoctonia* suggested that this parasite was *Rhizoctonia Napi* West, a fungus which was first identified upon *Brassica Napus* at Courtrai in Belgium.[1] Growth upon glucose agar was very vigorous and the same may be said of French bean[2] and oat juice[2] agar; on the two latter media a spore form was produced, the presence of which was particularly interesting in view of the recent work on the nature of *Rhizoctonia*. Modern evidence[3] has shown that the perfect stage of *Rhizoctonia* is, in some forms at least, a basidiomycete belonging to the genus *Corticium*. The spore form produced on French bean agar cultures of *R. Napi* has however no relation to *Corticium*. A fertile hypha of the mycelium bears numerous short branches which terminate in small spherical colourless conidia (Pl. V, figs. 2, 3, 4); sometimes the conidiophores branch again themselves, the ends of the secondary branches bearing the spores. In other cases the conidiophores are collected in a dense bushy terminal growth at the apex of a thick hypha. The whole morphology of this fertile stage strongly suggests the genus *Botrytis*. The short thick conidiophores are swollen at the base and taper to a point at the distal end bearing the spore. The spores are spherical hyaline bodies about 4μ in diameter in the examples illustrated in this paper, but cases have been observed in old cultures of $8\text{-}12\mu$ in diameter. The conidiophores vary in length from 6μ in the case of short unbranched individuals up to 24μ in the longer branched forms.

The spore form very rarely occurred on glucose agar, although numerous inoculations were made upon this medium from a mycelium containing fertile branches. A sub-culture upon French bean agar from a very young glucose agar culture, containing only about $\frac{1}{4}$ in growth of typical hyphæ, produced the spore

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[1] Saccardo, P. *Syll. Fung.*, Vol. XIV, p. 1176, 1899.

[2] Take 50 grms. of crushed French beans or oats and boil with 300 c.c. water for 1 hour and strain through a wire gauze. Dissolve 10 grms. of agar in 200 c.c. water, add decoction and heat to mix thoroughly.

[3] Rolfs, F. M. *Colorado Agric. Coll. Bull.* 70, 1902, *Bull.* 91, 1904.

Güssow, H. T. Bei trag zur kenntniss des Kartoffel Grindes, *Corticium vagum* B. C. var. *solani* Burt. *Zeits f. Pflanzenkrankh*, Vol. XVI, 1906.

Pethybridge, G. H. "Investigations on Potato Diseases." *Journ. Dept. Agric. Tech. Inst., Ireland*, Vol. XI, 1910-11.

Shaw, *loc. cit.*

form although the parent glucose culture never did so ; in only one case, that of a culture from a diseased gram plant to glucose agar, did the spore form occur on this medium. Moreover the fact that in culture it was possible to trace the fertile hyphæ in organic connection with hyphæ which had the typical branching of *Rhizoctonia* hyphæ (Pl. V, fig. 4) shows that the fertile form is part of the sclerotial fungus and not an impurity in culture as might have been suspected.

The growth of the fungus in agar culture continued quite vigorously up to the month of April. About this time it was noticed that the vigour of subcultures was declining, and in some cases no growth was obtained. At the same time the mycelium of older cultures began to turn black. Various devices were tried to induce the fungus to grow and it was finally discovered that temperature was the limiting factor. On transferring the cultures to a cool incubator at 23°C. growth continued in a normal manner ; the cultures were maintained in the cool incubator through the hot weather and were finally resumed in the open air in October. The average monthly temperature of the Mycological Laboratory during 1914 at Pusa was as follows :—

	°C.		°C.
January	.. 17	July	.. 31
February.. ..	.. 22	August	.. 28
March	.. 26	September ..	.. 29
April	.. 29	October ..	.. 27
May	.. 30	November ..	.. 23
June	.. 32	December ..	.. 18

The maximum temperature for the growth of ***R. Napi*** would therefore seem to be about 29°C.

In order to test the temperature relations of this fungus an experiment was made in which cultures were placed in incubators at different temperatures, *viz.*, 23°, 29°, 32°C. At the lower temperature 23°C. growth was strong both upon glucose agar and French bean agar. At 29°C growth was much weaker and sclerotial formation was slight; at 32°C growth was practically entirely inhibited. At the high temperatures the fungus turns a brown black colour in culture. This experiment therefore indicates 29°C as about the point at which the degree of temperature has a markedly inhibitory effect on the growth of *R. Napi*, while at 32°C. and upwards growth may be considered impossible. Since exposure to a temperature of 29°C. and upwards inhibits the growth of the fungus it is obvious that it can only occur as a serious parasite on the plains during the cold weather. On the other hand, it is by no means

certain that the heat of the hot weather and rains is sufficient to kill the fungus even when endured for a long period. During the month of June cultures ceased growth when kept for several weeks at the ordinary laboratory temperature (from 29·5—32°C.) but commenced growth again when placed in an incubator at 20°C. In a second experiment cultures were kept at 29°C. and 32°C. for 48 hours, at the expiration of which there was a very slight growth in the tube at 29°C., and were then transferred to a temperature of 23°C. and examined again after 48 hours. Growth was resumed strongly at the lower temperature but the tube which had been kept at 32°C. did not give such a good growth as that from 29°C.

The temperature of the soil in India has recently been the subject of investigation[1] and the following statement shows the approximate average temperatures throughout the year at depths of 2″, 6″, 12″, 18″, 24″ in the soil at Pusa.

Month	2″		6″		12″		18″		24″	
	Maximum °C.	Minimum °C.	Maximum °C.	Minimum °C.	Maximum °C.	Minimum °C.	Maximum °C.	Minimum °C.	Maximum °C.	Minimum °C.
January	22	11	19	14	16	17	17	17	18	18
February	27	15	23	17	21	19	20	20	20	20
March	33	17	28	20	25	22	23	23	23	23
April	36	22	31	24	28	26	27	27	27	27
May	39	28	35	30	32	31	31	31	31	31
June	39	29	35	31	34	32	33	33	33	33
July	35	28	33	29	30	30	31	31	31	31
August	35	28	32	29	31	31	31	31	31	31
September	35	27	33	29	31	30	30	23	31	31
October...	35	24	32	27	30	29	29	29	30	30
November	27	17	25	20	23	22	23	23	24	24
December	23	12	20	15	18	18	19	19	20	20

It is clear from the above table that *R. Napi* is incapable of growth in the first 24 inches of Pusa soil from May to October, and indeed the high temperatures experienced in the upper layers of the soil might suffice to kill the fungus, but, from the facts detailed above, it is evident that the fungus might be capable of withstanding prolonged exposure to the temperatures which occur at depths below 18″ from the surface, and thus surviving until the next cold weather when vegetative growth is again possible. We must be cautious however in making a comparison between a fungus enduring a high temperature in a culture tube with an abundant supply of food and moisture and one meeting the same degree of temperature in the Indian soil under natural conditions when extremes of heat and drought frequently coincide.

[1] Leather J. W. "Soil Temperatures." *Mem. Dept. Agr. India, Chemical series*, Vol. IV, No. 2, 1915.

Infections from agar culture, upon mustard (Pl. I, fig. 1) and gram plants were invariably successful, the death of the host taking place in every case ; the fungus formed a copious growth on the exterior of the stem, as well as penetrating within, and numerous sclerotia, together with the spore stage, were formed. From the diseased plants the fungus was again isolated in pure cultures where it again produced the spore form.

Rhizoctonia destruens Tass.

This fungus was first noticed in July, 1912, when it appeared on rotting potato tubers sent from storage at Bankipore. The diseased potatoes were rather soft and showed thick white strands of mycelium and brown sclerotia on the external surface. The tubers were incubated in a moist chamber and gave a very copious growth of white hyphæ (Pl. IV, fig. 2) ; the hyphæ showed a tendency to become united in strands which made the mycelium very tough and gave it a fairly characteristic appearance. The branching of the hyphæ followed the characteristic form of *Rhizoctonia* and clamp connections were very numerous. The sclerotia were formed in abundance, were light brown in colour and of fairly regular spherical shape about 1-2 mm. (Pl. IV, figs. 2, 3) in diameter. In section the sclerotia showed a white interior, surrounded by a thin brown outer layer consisting of two or three hyphæ divided up into rectangular thick walled cells. At the time when this parasite was first collected it was not identified with any certainty ; the fungus was obviously not the same as the *R. Solani* Kühn, which had been found previously on rotting potatoes, but the matter was finally decided in February, 1913 when specimens of *Delphinium*, suffering from the same fungus, were received from Alipore, Calcutta. The fungus was obtained in culture from the *Delphinium* and showed a strict agreement with that on the potato. A comparison of the parasite with the description of *R. destruens*[1] Tass., first described by Tassi on *Delphinium* in the botanical garden at Siena in Italy, left no doubt as to the identity of the fungus. Infections from glucose agar upon healthy uninjured Delphinium plants (Pl. III, fig. 1) gave 100% of deaths in a few days. Since making the above observations the fungus has appeared as a virulent parasite of different crops in various parts of India.

(1) Betel vine (*Piper betle*) is seriously damaged both in Bengal and Bombay (Pl. III, fig. 2). In Bengal the disease occurs in the vicinity of Bogra, during the rainy season, and in Bombay it is common in the district of Nasik and also occurs in Dharwar and Belgaum. The fungus attacks the plants at about the level of the collar (Pl. III, fig. 2) and below the

[1] Saccardo, P. *Syll. Fung.*, Vol. XVI, p. 1109.

soil, where it sets up an extensive rot in the stem swiftly leading to the death of the whole vegetative portion above ground. The whole of the rotted portions of the plant become covered with a thick weft of hyphæ with numerous sclerotia. From a diseased plant it is easy to obtain the fungus in culture; its form in culture (Pl. VI, fig. 1) does not differ from that on the host and agrees with that seen previously upon potato and *Delphinium*. Cultures of the fungus from Bombay Presidency sometimes showed the sclerotia slightly stalked and the same thing was also noticed on diseased betel vines from Bogra. This agrees exactly with Tassi's original description as given in Saccardo "Tuberculis ..... interdum minute umbilicatis." Sometimes this feature is so marked that the sclerotia take the form of club shaped bodies, recalling the sclerotia of *Xylaria*, in this state they often remain white in colour and never become brown; occasionally the sclerotia become fastened together in clumps—a common feature in this genus. Inoculations from agar cultures upon betel vine were very successful both at Pusa and at Poona, the infected plants dying in every case with a copious development of the fungus. No trace of a perfect stage was ever found. A basidiomycete, identified with *Dædalea*, was found closely associated with *Rhizoctonia destruens* on betel vine, and on stems of *Sesbania ægyptica*, grown as supports for the vine, both in Nasik and Belgaum. But no organic connection could be traced between the two nor did successful inoculation with *R. destruens* on *Sesbania* succeed in producing the *Dædalea*.

(2) Potato (*Solanum tuberosum*). As already mentioned the potato is liable to disease owing to this fungus (Pl. IV, figs. 2, 3). In Bihar the parasite has appeared chiefly as a rot of stored potato, but in the vicinity of Poona it is a frequent cause of trouble to the growing crop. The mode of attack is exactly similar to that observed in the betel vine, the portion of the stem at the ground level, and the subterranean parts of the plant, becoming covered with a weft of hyphæ with sclerotia (Pl. IV, fig. 3). This is followed by the rotting of these parts and by the drying and browning of the leaves; the attack is usually late about six weeks after the planting of the sets. The tubers from diseased plants are apparently sound although smaller than those from healthy plants but cases have been found in which apparently sound tubers from diseased plants have been infected with the mycelium through their attachment to the parent stem. The experience with stored potatoes in Bihar shows that tubers from a diseased crop are probably infected with the fungus. The use of a wash of standard corrosive sublimate, combined with proper precautions in storage, would certainly go far towards lessening this disease in store.

Inoculations upon potato tubers were uniformly successful, when the infection was made upon the cut surface of a tuber. In unwounded tubers although the fungus spread over the surface of the tuber the external corky covering was sufficient in many cases to prevent penetration. Sometimes, however, penetration took place through the eyes, or even through the lenticels. The same results were obtained whether the fungus used for inoculation had been isolated from a diseased potato or from the betel vine.

(3) Suran (*Amorphophallus campanulatus*). This crop is, in Poona, frequently damaged by *R. destruens*; the corms rot completely in the soil and the shoots die later (Pl. IV, fig. 1). In culture the fungus from suran is indistinguishable from that of the other hosts and inoculations gave similar results to those on the potato. In every case in which the outer surface of the corm was wounded before making the infection the fungus established itself rapidly and the death of the host soon took place. If, however, the outer corky surface of the corm were unbroken successful infections were rare. Cross inoculations from potato and betel vine yielded the same results.

(4) Lucerne (*Medicago sativa*) has been found suffering from *R. destruens* at Surat, but the damage was not extensive and the parasite is unusual on this host. The fungus attacks the plants at the collar, setting up a rot of the stem and leading to rapid death of the aerial portion.

(5) Groundnut (*Arachis hypogœa*) has also been observed to suffer from *R. destruens* when sown on land which had previously borne a *Rhizoctonia* infected potato crop.

(6) Rice (*Oryza sativa*). Although *R. destruens* is not at present known as a parasite of rice in India it has been collected on this host in Java during the last two years.

In November, 1913, some rice-seedlings, with brown mustard-seedlike sclerotia formed on them at the collar, were collected at Buitenzorg, Java. The rice-seedlings were dying in large numbers, presumably as a result of the attack of this sclerotium-producing fungus.

The sclerotia were used for obtaining pure cultures on glucose-peptone-agar medium, with the result that a fungus was obtained which was indistinguishable in any particular from the *Rhizoctonia destruens* on betel vine and suran; no spore form has been obtained though the fungus has been in cultivation for over a year. Some inoculations were carried out with pure cultures from the rice sclerotia on suran, potato and rice as hosts. The two former hosts were successfully infected but the results with rice were inconclusive.

Several sclerotia-forming fungi are known as parasites of the rice plant[1] but up to now all have been members of the genus *Sclerotium*, namely, *Sclerotium Oryzæ* Catt., *Sclerotium glumale* Ces. and *Sclerotium irregulare* Miy., and can easily be distinguished from *R. destruens* Tass. The most recent work on a sclerotial parasite of paddy is that of Van der Wolk[2] from Java. From this author's description and figures it is not possible to form any clear picture of the characteristics of the sclerotia and mycelium of his fungus, which he has elevated into a new species under the name *Sclerotium omnivorum*. Van der Wolk also states that two spore forms, one a species of *Ascobolus*, were connected with his sclerotial fungus; as however the evidence on which this was based was derived from cultures consisting of pans of rotting vegetable matter, from which it is admitted that saprophytic bacteria were not excluded, it is difficult to regard the author's point as satisfactorily established.

There is no evidence of any specialization in the parasitism of *R. destruens*. Cross inoculations with the form from one host plant on to another host were invariably successful. *Delphinium* is susceptible to the attack of all forms and successful inoculations on unwounded plants were obtained with *R. destruens* from betel vine, potato and lucerne. On potato and suran the fungus appears to be a weaker parasite than on betel vine and *Delphinium*: the former hosts requiring a wound in their superficial tissues before the fungus can penetrate. Jowar seedlings are also attacked by *R. destruens* and inoculations in the laboratory gave 60% of deaths; moreover a basidiomycete resembling a *Hypochnus* was found associated with the *Rhizoctonia* on the jowar. In view of the connection between other species of *Rhizoctonia* and the genus *Corticium*[3] this result was very suggestive, but, unfortunately, all attempts to repeat this experiment from the point of view of producing the basidiomycete, were unsuccessful.

Methods of Treatment against *Rhizoctonia* Disease.

Fungi such as *Rhizoctonia* possess a method of perennating from one season to another by means of their sclerotia. It is therefore important to obtain some idea as to the longevity of the sclerotia. The sclerotia of *R. destruens*

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[1] Shaw, F. J. F. "Sclerotial Disease of Rice." *Mem. Dept. Agric., India, Bot. Ser.*, Vol. VI, No. 2, 1913.

Miyake, I. Studien über der Pilze der Reispflanze in Japan. *Journ. College of Agric., Tokio*, 1910.

[2] Van der Wolk, P. C. *Rhizostilbella rubra* (n. gen. n. spec.) a by-fruit form of *Ascobolus parasitiens* (nov. spec.) and its connection with the "Sclerotium disease" of certain tropical cultivated plants (*Sclerotium omnivorum*). *Mycologische Centralblatt* Bd. IV, Hft. 5, June, 1914.

[3] Rolfs, Güssow, Pethybridge, Shaw. *loc. cit.*

have been found to germinate when about sixteen months old, but sclerotia kept much longer than this generally failed to germinate. This suggests that rotation of crops, and avoiding a *Rhizoctonia*-susceptible host for a few seasons might be a useful practice, but extensive experiments are necessary to establish the utility of this method of starving the fungus out of the soil. Methods of sterilizing the soil which may be practicable in the laboratory or seed nursery are hardly ever possible in general agriculture; since, however, *R. destruens* is a parasite on *Delphinium* and *Dianthus* it might be worth using a soil fungicide in such cases.

Various methods have been used to sterilize soil against *Rhizoctonia*. In the prevention of damping off of coniferous seedlings, due to *Rhizoctonia* or *Pythium*, the use of commercial sulphuric acid as a soil fungicide is recommended by a number of authors[1]; the best strength seems to be a solution of 1 oz. in 1 gallon of water applied at the rate of 3/16 oz. per square foot. Formalin is often used as a soil fungicide,[2] the strength of the solution is commercial formalin 1 pt. water 50 pts. at the rate of $\frac{1}{2}$ gallon per square foot, and Salmon[3] and Eriksson[4] both suggest the use of a solution of phenol, but sterilization by heat is undoubtedly one of the most efficient methods. In the case of seedling beds steam sterilization by the inverted pan method is strongly recommended by Johnson[2], but the less troublesome and expensive formalin treatment would probably be preferred by most people. A marked instance of the sterilizing effect obtained by heating the surface of the soil is provided in the raising of tobacco seedlings in Pusa.[5] It has been found that in seedling beds which have been so treated the plants are practically immune from a destructive damping off due to a fungus which, under certain conditions, does great damage. There is reason to believe that this particular disease is due to *R. Solani* Kühn. In all cases of sterilization of soil by formalin or by steam it is necessary to remember the secondary effects which may arise

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1 Spaulding, P. "Treatment of damping off in Coniferous seedlings." *U. S. Dept. Agric. Bureau of Plant Industry, Circ.* No. 4, 1908.

Hartley, Carl. "Use of soil fungicides to prevent damping off in coniferous seedlings." *Proc. Soc. Amer. Foresters*, Vol. VIII, 1912.

Kræmer, H. "Dilute sulphuric acid as a Fungicide." *Proc. Amer. Phil. Soc.*, Vol. XLV, 1906.

2 Johnson, J. "Control of damping off in Plant beds." *Agric. Expt. Sta. Univ. Wisconsin, Bull.* 31, 1914.

3 Salmon, E. *Gardeners' Chronicle* 44, p. 1, 1908.

4 Eriksson, J. Einige Studien über Wurzeltöter. *Centralblatt. f. Bakt. Parasit. Infektionskrank*, Vol. X, 1903.

5 Howard, A., and Howard, G. L. C. "Improvement of Tobacco Cultivation in Bihar." *Pull.* No. 50, *Agric. Res. Inst., Pusa.*

in soil so treated.[1] In the cultivation of paddy in some parts of the Bombay Presidency the practice of " rabbing,"[2] that is of burning the surface layers of the soil by spreading fire wood and rubbish and igniting it, would not be without some sterilizing effect and might perhaps be extended with advantage to the preparation of land for betel vine.

With crops such as potato and suran, in which portions of subterranean stems are used for seed purposes, there is always the possibility of sowing tubers or corms which have sclerotia adhering to their surface. In these cases treating the seed with some fungicide is a cheap and efficient method of lessening the chances of infection. The effect of corrosive sublimate and of formalin as a seed dip was determined by steeping sclerotia of *R. destruens* for 2½ hours in each and then trying to germinate them on wet blotting paper and in culture tubes.

Fungicides	Strength	Result
Corrosive Sublimate ...	1·0 %	No sclerotia germinated.
,, ...	0·1 ,,	,, ,, ,,
Formalin	0·5 ,,	,, ,, ,,
,, ...	0·25 ,,	Many germinated after 6 days.
,, ...	0·12 ,,	All germinated after 2 days.

The number of sclerotia used in each case was about 50. In the controls with untreated sclerotia all the sclerotia germinated in two days. Solutions of copper sulphate proved unreliable as a fungicide in the case of *R. destruens*, and sclerotia which had been immersed for 2 hours in 5, 2·5, 1·25 and 0·5% copper sulphate all germinated in from 24 to 48 hours. The failure of the copper sulphate may have been due to the inability of this solution to penetrate to the interior of the sclerotium ; accordingly, the experiment was repeated with the difference that the vessels containing sclerotia immersed in solutions of copper sulphate were placed under an air pump, at the same time of course a control was carried out under normal atmospheric pressure. Under reduced pressure 5, 2·5, 1·2% copper sulphate prevented the germination of the sclerotia, but 0·5% was not strong enough, and, in this case, germination took place. In the controls under normal atmospheric pressure germination took place after treatment with 0·5 and 1·25% but not after treatment with 5 or 2·5%. This result differed from that obtained in the first experiment, in which even the sclerotia which had been immersed in 5% $CuSo_4$ germinated,

[1] Russell & Pethybridge. *Journ. Board. Agric., London*, XVIII, p. 809, 1912.

[2] Mann, H. H., Joshi, N. V., & Kanitkar. N. V. " Rab system of rice cultivation in Western India." *Mem. Dept. Agric., India, Chemical series*, Vol. II, No. 3, Feb. 1912.

but the relatively greater efficiency of the copper sulphate in the second experiment was probably due to the fact that the sclerotia used were rather younger and possibly did not possess such a well developed outer protective layer.

Strength of Copper Sulph.	Old sclerotia Normal atmospheric pressure	Young sclerotia Normal atmospheric pressure	Young sclerotia Under vacuum
5·0 % ...	Germinated in 48 hours	No germination	No germination
2·5 ,, ...	,, ,, ,, ,,	,, ,,	,, ,,
1·2 ,, ...	,, ,, 24 hours	Germination in 48 hours.	,, ,,
0·5 ,, ...	,, ,, ,, ,,	,, ,,	Very slight germination.

It is evident from these experiments that copper sulphate in strengths below 5% can hardly be considered a reliable fungicide against *R. destruens* and on the whole treatment with a solution of corrosive sublimate is probably the most efficient method of killing such sclerotia as may adhere to a potato tuber or suran corm. It should always be remembered that treatment with a fungicide is intended as an assistance to, and not as a substitute for, hygienic methods in storing.

This result agrees with the conclusions of American investigators who have done extensive field experiments on the treatment of potato tubers against *Rhizoctonia* disease. Rolfs[1] found that the corrosive sublimate treatment improved the appearance of the crop and gave marked gains when the treated seed was planted on new lands, proving much more efficient than treatment with formalin. The work of Güssow[2] and of McAlpine[3] also bore out this conclusion, while Gloyer[4] has found that neither formaldehyde gas nor formaldehyde solution can be depended upon to kill *Rhizoctonia* sclerotia, but that treatment with 1-2000 solution of corrosive sublimate is an efficient fungicide against *Rhizoctonia*; with formaldehyde gas according to Gloyer there even appears to be some danger of injuring the potatoes. On the other hand other investigators[5] recommend the formalin treatment or a lime sulphur wash as a fungicide against the "dry rot" of Irish potatoes which is due to *Fusarium tuberivorum*. It is unfortunate that these workers did not make a

1 Rolfs, F. M. *loc. cit.*

2 Güssow H. T. *Canada Expt. Farm Reports*, 1912, p. 209.

3 McAlpine, D. *Fungus Diseases of Potato in Australia*, 1911.

4 Gloyer, W. O. *New York Agric. Expt Sta. Bull.* 369, 570, 1913.

5 E. Wilcox, G. Link & Venus W. Pool. "Dry Rot of Irish potato." *Bull.* 1 *Agric. Expt. Sta. Nebraska*, 1913.

trial of corrosive sublimate as it would be a great advantage to obtain a method of treatment which would be equally efficient against both *Fusarium* and *Rhizoctonia*. In this connection we must note that yet another fungicide is recommended by Sherbakoff [1] in the treatment of potato scab. Sherbakoff states that mixing sulphur with the soil, at the rate of 450—900 lbs. per acre, has a marked effect in checking this disease but it is by no means certain that the after effects of the sulphur on the fertility of the soil are not more to be dreaded than the loss due to any disease. On the other hand, a seed dressing of napthalene [2] appears to be an efficient and harmless remedy against sore shin in cotton.

There is an apparent discrepancy in the above account between the results of those investigators (Johnson), who found formalin an effective soil fungicide and those (Rolfs, Güssow, Gloyer) who found that it was not so efficient as a disinfectant for potato tubers. It is necessary to bear in mind that Johnson worked with a solution of 1 pt. formalin in 50 pts. water and that Rolfs and Gloyer used a solution of 1 pt. formalin in 240 pts. water. Our experiments have shown that formalin solution of a strength below 0·5% cannot be trusted to kill sclerotia of *R. destruens* but that corrosive sublimate in strengths of 1 pt. in 1000 pts. water is a reliable fungicide. In recommending mercuric chloride as a disinfectant for potato tubers before storing it is necessary to bear in mind that potatoes so treated cannot be safely used for human consumption and the treatment is therefore only suitable in the case of tubers which are intended for seed purposes. Güssow and Shutt [3] have shown that 3 lbs. of potato tubers (13 tubers) treated for 3 hours with 1-2000 corrosive sublimate solution will take up from the solution 0·05 gm. of mercuric bichloride, which is six times the maximum official dose in medicine; potatoes which have been so treated must therefore be regarded as non-edible. The very material reduction in strength of the solution shows that the solution must be renewed fairly often, it is possible that the neglect of this precaution accounts for the supposed failure of corrosive sublimate to prevent the reappearance of disease in certain cases.

Systematic.

In the case of a genus such as *Rhizoctonia*, which is identified by its vegetative characters, it is always possible that we are dealing with an artificial group of which the different species are vegetative stages of widely separate

[1] Sherbakoff, C. D. *Cornell Univ. Agric. Expt. Sta. Bull.* 350, 1914.

[2] Balls, W. L. "Physiology of simple parasite." *Year-book Khed. Agric. Soc.*, 1905-06.

[3] Güssow, H. T. *Canada Expt. Farm Reports*, 1912, pp. 200—2.

fungi and in which the morphological similarities between the species are accidental; the study of *R. Napi* appears to furnish an example of this.

A disease of *Brassica* was discovered by Frank[1] in 1879 which, in its effects upon the host plant, appears to be indistinguishable from that which we have attributed to *R. Napi* West. The fungus consisted of a dense white mycelium with large black sclerotia which, in section, showed a white interior surrounded by a black outer layer; the spore form was the well-known *Botrytis cinerea* Pers. Comparing the description of the sclerotia of *R. Napi* West with the sclerotia of Frank's fungus one is forced to the conclusion that the two fungi are the same and that they are identical with the fungus described in this paper. It follows from this that the spore form which we obtained is *Botrytis cinerea* Pers. and the criticism will at once arise that this form (Pl. V, figs. 2, 4) differs widely from that which normally occurs in the species. It has, however, been shown that the morphology of *B. cinerea* is closely dependent on the composition of the nutrient medium[2] and a variation of the normal form is known (Beauverie et Guilliermond, p. 281, fig. 6) which agrees exactly with that figured on Plate V of this paper. Additional proof is furnished by the fact that another variation from the normal form of *Botrytis*, in which the swollen ends of hyphæ branch in an umbellate manner (shown in Fig. 1 Beauverie et Guilliermond) is also common in our cultures. The normal method of branching which occurs in the hyphæ figured in this illustration (Fig. 1, Beauverie et Guilliermond) is almost typical of *Rhizoctonia*. Frank states that the sclerotia of *Botrytis cinerea* will germinate in damp sand and give rise to the apothecia of *Sclerotinia Libertiana* Fuck.; up to the present sclerotia of our fungus have not produced any apothecia. Smith[3] throws doubt on the connection between *B. cinerea* and *S. Libertiana* but Istvanffi appears to have obtained satisfactory evidence that they are different stages of the same fungus.

There is a large and convincing body of evidence (Rolfs, Güssow, Shaw) which shows that the fertile stage of at least one species of *Rhizoctonia* is a basidiomycete of the genus *Corticium*; from the present paper, however, it appears that *R. Napi* has its fertile stage in a totally different group and that its inclusion in the genus is an error. In brief this species furnishes an instance of the fact that the genus *Rhizoctonia*, as constituted on purely vegetative

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[1] Frank, A. B.—Kampfluch gegen die Schädlinge unserer Feldfruchte. Berlin, 1897, p. 276.

[2] Beauverie, J. et Guilliermond, A.—Etude sur le structure du *Botrytis cinerea*. *Centralbl. Bakt. Parasit. Infektionskrank*, Vol. X, 1903.

[3] Smith, R. E. *Botrytis* and *Sclerotinia*. *Bot. Gaz.* June, 1900.

„ „ Parasitism of *Botrytis cinerea*. *Bot. Gaz.* June, 1902.

characters, is an artificial group. It will probably be better to limit the use of the name to the sclerotial stages of those fungi with a perfect stage in the genus *Corticium*.

The point in which we differ widely from other workers on this genus is in the facility with which they have assumed that the *Rhizoctonia* on potato is always to be identified with *R. Solani* Kühn. The practice of multiplying the species of a genus according to the number of hosts on which it is parasitic has been responsible, in some genera, for a great deal of unnecessary complexity, but the converse process whereby different species of a genus, all parasitic on the same host, are referred to under a single specific name, derived from the host, is equally confusing. The naming of a species after the host plant on which it is parasitic (*e.g. R. Solani* on *Solanum tuberosum*) is apt to give rise to an attitude of mind which automatically refers that genus, when parasitic on that particular host, always to the one species. In a previous publication we have given reasons for believing that the fungus *R. Solani* Kühn is identical with the form of *Rhizoctonia* having small black sclerotia, resembling perithecia (Pl. VI, fig. 2), and is distinct from the species with large brownish sclerotia which has a fertile stage in the genus *Corticium*. *R. destruens* Tass. is also a distinct and separate species, its parasitism on the potato and on lucerne being merely one phase of its activities, while *R. Napi*, with its spore form, is a totally different organism.

The species of *Rhizoctonia* which are known in India are therefore as follows :—

(1) *R. Napi* West. Sclerotia large irregular bodies about 3-12 mm. broad, black on outside, flesh coloured on interior. This species should be removed from the genus *Rhizoctonia*, as it appears to be merely a synonym for *Botrytis*.

(2) *R. destruens* Tass. Sclerotia roughly spherical about 1-2 mm. in diameter, brown on outside, white within.

(3) *R. Solani* Kühn. Sclerotia small black bodies resembling perithecia of an ascomycete. *R. Medicaginis* D. C. is almost certainly the same fungus.

(4) *Rhizoctonia* sp. This form has *Corticium vagum* as its perfect stage, its parasitism in India has been described in a previous communication.[1] The sclerotia are irregular structures 2-5 mm. in diameters dark brown colour both on exterior and within (cf.—*R. destruens*), the mycelium when old becomes of a reddish brown

[1] Shaw, F. J. F. *loc. cit.*

colour. This last form, which in India is parasitic upon ground-nut and cowpea, and which bears the very closest resemblance to the *Hypochnus* described by Stevens and Hall[1] as parasitic upon quince, appears to be that which Rolfs, Güssow, Pethybridge[2] have identified with *R. Solani* Kühn ; we have given reasons elsewhere for differing from this identification and indeed suggested that it might be *R. destruens* Tass., a supposition now obviously incorrect.

The following is a list of the principal host plants of *Rhizoctonia* in India :—

R. Solani Kühn.	*R. destruens* Tass.	*R. Napi* West	*Corticium vagum* B. & C.
on	on	on	on
Alysicarpus	*Arachis hypogaea*	*Amaranthus tristis*	*Arachis hypogaea*
Arachis hypogaea	*Amorphophallus campanulatus*	*Argemone mexicana*	*Solanum tuberosum*
Carica papaya	*Delphinium*	*Avena sativa*	*Trichosanthes cucumerina*
Citrullus vulgaris	*Dianthus*	*Beta bengalensis*	*Vigna Catiang*
Corchorus capsularis	*Medicago sativa*	*Brassica campestris* var *glauca*	
Crotalaria juncea	*Piper betle*	*Calamintha*	
Cucurbita maxima	*Solanum tuberosum*	*Cannabis sativa*	
Dolichos biflorus		*Chenopodium album*	
Dolichos Lablab		*Cicer arietinum*	
Gossypium		*Cnicus arvensis*	
Hibiscus cannabinus		*Fumaria parviflora*	
Lycopersicum esculentum		*Hordeum vulgare*	
Medicago sativa		*Lathyrus sativus*	
Morus alba		*Lens esculenta*	
Nicotiana Tabacum		*Leucas*	
Phaseolus lunatus		*Linum usitatissimum*	
Phaseolus Mungo var *radiatus*		*Medicago lupulina*	
Sesamum indicum		*Pisum sativum*	
Solanum Melongena		*Scoparia dulcis*	
Solanum tuberosum		*Triticum vulgare*	
Vigna Catiang		*Vicia hirsuta*	

Agricultural Research Institute, Pusa,
March, 1915.

[1] Stevens and Hall. "Hypochnose of Pomaceous Fruits." *Ann. Mycol.* VII, 1909.
[2] Rolfs, Güssow, Pethybridge. *loc. cit.*

DESCRIPTION OF PLATES.

Plate I.

Fig. 1. Gram plants infected with *R. Napi* West. The infected portion of the plant is light in colour, a growth of mycelium is visible on portions of pods and stem marked " a ".

„ 2. Mustard plants infected with *R. Napi* West. One plant has collapsed, the white fluffy growth of mycelium on stems and leaves is clearly visible. Infected portions, marked " a " show white mycelium.

Plate II.

Fig. 1. *Cannabis sativa* infected with *R. Napi* West., infected portion marked " a " shows white mycelium.

„ 2. Section of sclerotium of *R. Napi* West.

„ 3. The same showing the outer layer of thick-walled blackish cells.

Plate III.

Fig. 1. *Delphinium* infected with *R. destruens* Tass. The plant on the right has collapsed at point of infection; the manner in which the mycelium forms white strands both on the soil and on the plants is clearly shown in this and the next photo. It is indicated by letter " a ".

„ 2. *Piper betle* infected with *R. destruens* Tass. Note the mycelium (" a ") and sclerotia spreading on the soil.

Plate IV.

Fig. 1. Corms of suran infected with *R. destruens* Tass.—White growth of fungus (" a ") is easily visible.

„ 2. Potato tuber covered with growth of *R. destruens* Tass.

„ 3. Potato stem covered with growth of *R. destruens*. Sclerotia are very numerous in both these photos of diseased potato.

Plate V.

Fig. 1. Hypha of *R. Napi* West. × 1000.
„ 2. Fertile hypha with spores—*R. Napi*. × 250.
„ 3. Conidiophore and conidia—*R. Napi*. × 1000.
„ 4. Fertile hypha in organic connection with hypha of *R. Napi* West. × 250.
„ 5. Section stem of *Delphinium* infected with *R. destruens* Tass. × 375.

Plate VI.

Fig. 1. Culture of *R. destruens* Tass. from betel vine.
„ 2. „ „ *R. Solani* Kühn from potato.
„ 3. „ „ *R. Napi* West from mustard.
„ 4. „ „ Sclerotial stage of *Corticium vagum* B. & C. from cowpea.

Fig. 1.

Fig. 2.

PLATE I.

Fig. 1.

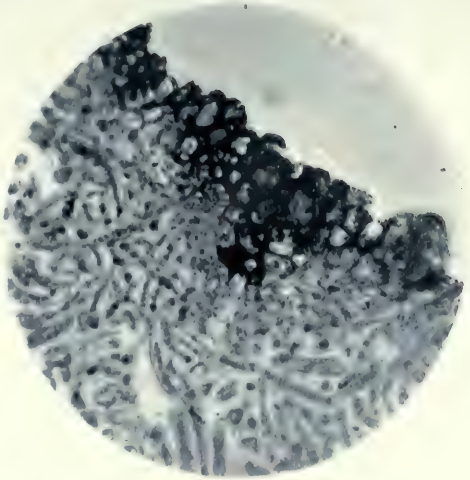


Fig. 2.

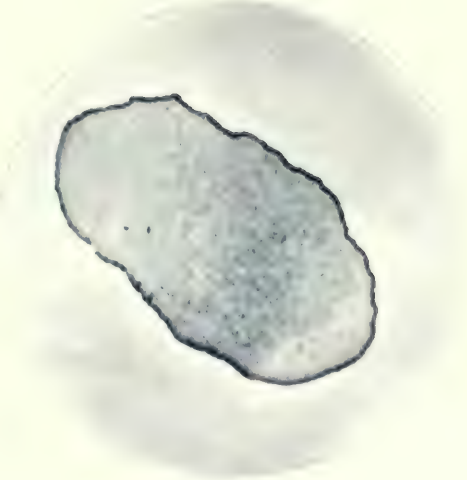


Fig. 3.

Fig. 1.

Fig. 2.

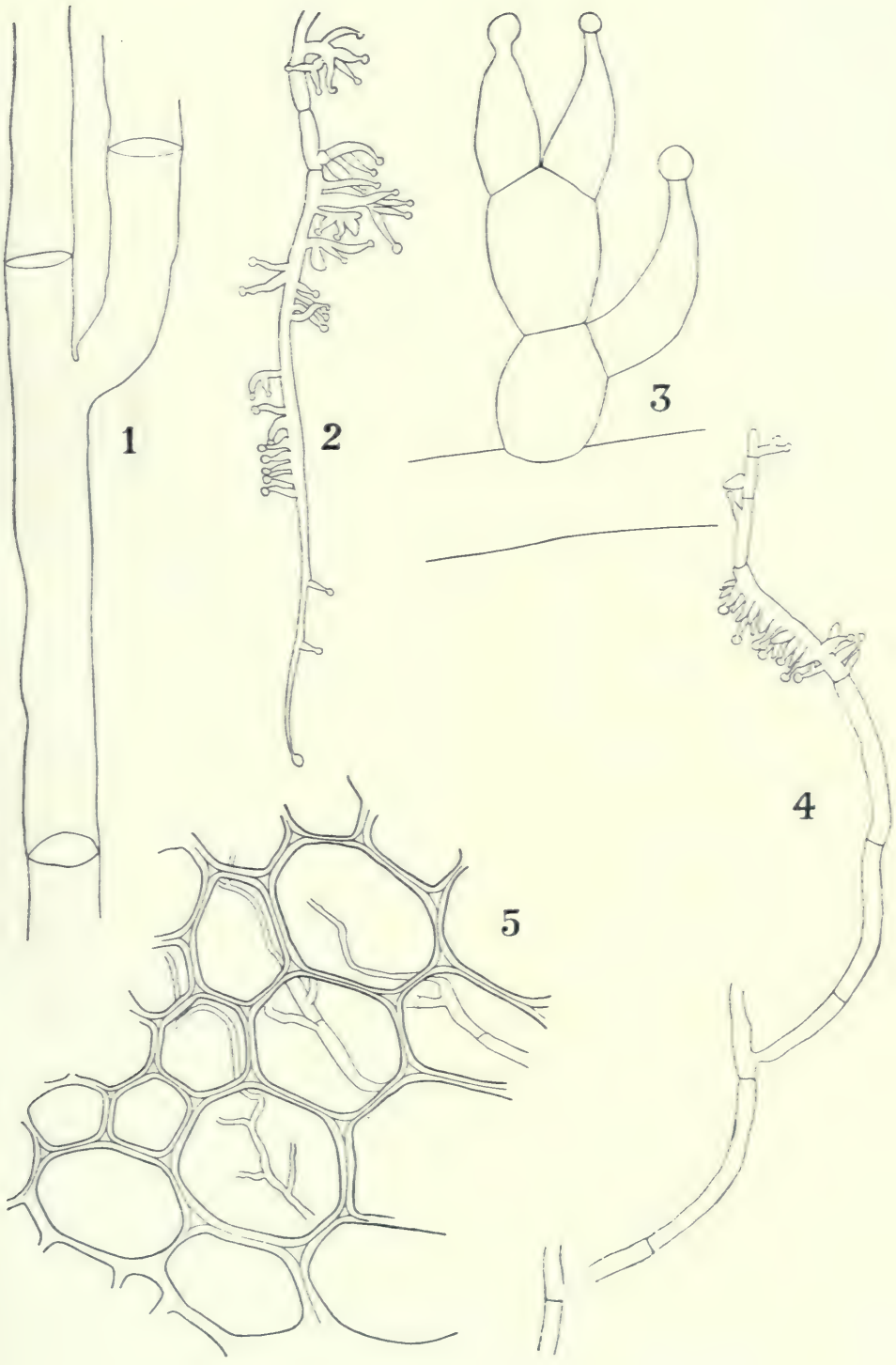
PLATE III.

Fig. 1

Fig. 3

Fig. 2

PLATE IV.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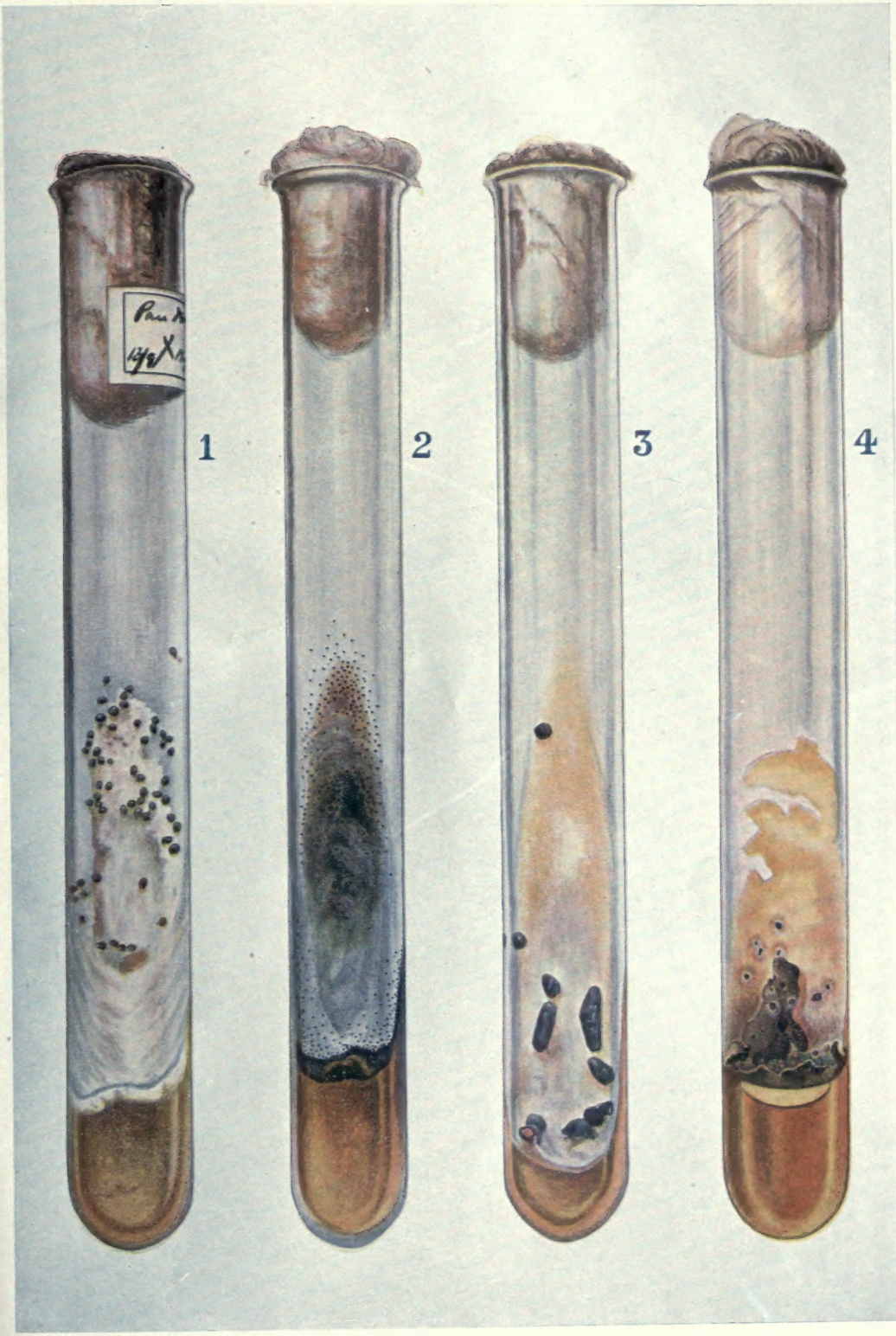
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